BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Inborn errors of the Krebs cycle: a group of unusual mitochondrial diseases in human

P. Rustin *, T. Bourgeron, B. Parfait, D. Chretien, A. Munnich, A. Rötig

Unité de Recherches sur les Handicaps Génétiques de l'Enfant, INSERM U-393, Hôpital des Enfants-Malades, 149, rue de Sèvres, 75743 Paris Cedex 15, France

Received 7 February 1997; revised 4 April 1997; accepted 10 April 1997

Abstract

Krebs cycle disorders constitute a group of rare human diseases which present an amazing complexity considering our current knowledge on the Krebs cycle function and biogenesis. Acting as a turntable of cell metabolism, it is ubiquitously distributed in the organism and its enzyme components encoded by supposedly typical house-keeping genes. However, the investigation of patients presenting specific defects of Krebs cycle enzymes, resulting from deleterious mutations of the considered genes, leads to reconsider this simple envision by revealing organ-specific impairments, mostly affecting neuromuscular system. This often leaves aside organs the metabolism of which strongly depends on mitochondrial energy metabolism as well, such as heart, kidney or liver. Additionally, in some patients, a complex pattern of tissue-specific enzyme defect was also observed. The lack of functional additional copies of Krebs cycle genes suggests that the complex expression pattern should be ascribed to tissue-specific regulations of transcriptional and/or translational activities, together with a variable cell adaptability to Krebs cycle functional defects. © 1997 Elsevier Science B.V.

1. Introduction

1992: 'This pathway (i.e. the Krebs cycle) is so crucial to the metabolism of living cells that any significant defect is incompatible with life' [1]. Accordingly, enzyme defects affecting the Krebs cycle, also known as the tricarboxylic acid cycle (TCAC), were for long considered as highly unlikely. Thus, even the disclosure of a high fumaric aciduria in patients was not sufficient to prompt authors (in the eighties) to measure fumarase activity: 'since it is very probable that the concomitant failure of oxidative metabolism associated with the TCAC would have catastrophic physiological and clinical consequences' [2].

However, although rare diseases (less than 25 cases reported in the literature), defects affecting one or more of the TCAC enzymes have been now convincingly established. For the sake of comparison, on 1300 young patients (0–15 yr) investigated in our laboratory, we identified 6 cases with a TCAC enzyme defect compared to 220 cases with a respiratory chain enzyme defect. In the three last years, the molecular bases were elucidated in the case of two of

* Corresponding author. Fax: +33 1 47348514. E-mail: rustin@necker.fr

PII S0925-4439(97)00035-5

these inherited metabolic diseases, namely fumarase and succinate dehydrogenase deficiencies [3,4].

2. A multi-functional cycle

Since its formalization by Hans Adolf Krebs in 1937, the TCAC, also known as the Krebs cycle, has proved to be a major turntable of cell metabolism [5]. The conversion of the reducing power accumulated into carbon compounds to the respiratory chain-usable reduced coenzymes, NADH and $FADH_2$, constitutes the major function of the TCAC. However, it also ensures a central role in the intermediary metabolism through the breakdown of acetyl-CoA and through the interconversion of carbon skeletons required for several anaplerotic pathways (Fig. 1). Moreover, TCAC should also be considered as a water splitting process generating oxygen for glucose oxidation and CO_2 production [6]. Two reactions of the TCAC consume one H_2O molecule each furnishing oxygen for CO_2 generation and oxidative reac-

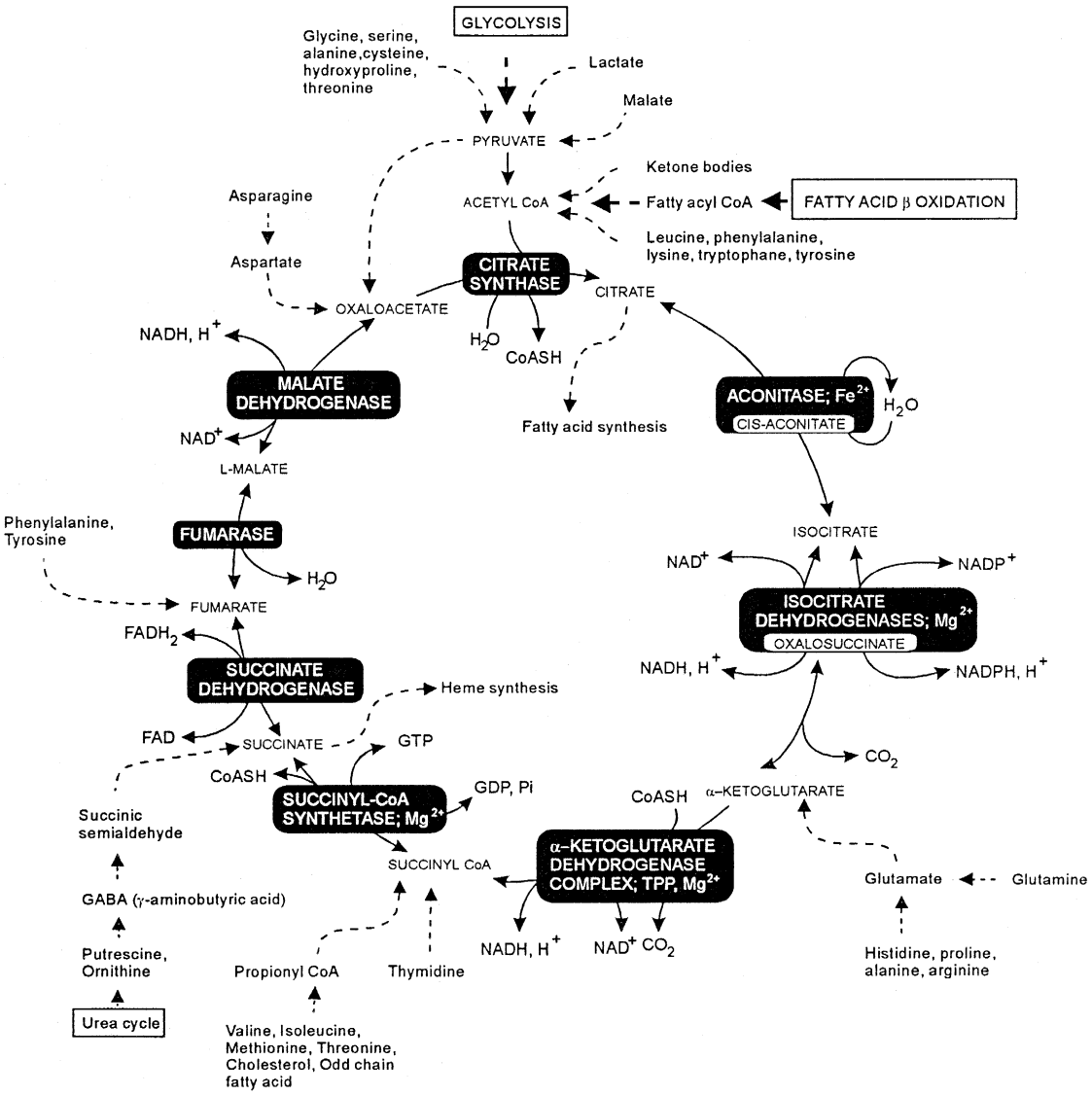

Fig. 1. Enzymes, reactions and integration in cell metabolic pathways of the Krebs cycle.

tions, namely the combination between acetyl-CoA and oxaloacetate to form citrate and the formation of malate from fumarate. Atmospheric oxygen is only used for the reoxidation of reduced coenzymes by the respiratory chain.

The TCAC is constituted by a series of 8 biochemical reactions that lead to the progressive oxidative decarboxylation of the acetyl CoA, mostly resulting from the activity of the pyruvate dehydrogenase (Fig. 1). Beside pyruvate from the glycolysis, the TCAC can be fed by carbon compounds derived from fatty acid degradation or from the several amino-acids (glutamate, alanine, etc.). This traditional view of the TCAC functioning has nevertheless been challenged

Table 1
The enzymes of the Krebs cycle

Mitochondrial TCAC enzymes		Alternative terms	Chromosomal location (D-segment) (available human cDNA)	Cytosolic isoenzyme
Citrate synthase (E.C. 4.1.3.7 Homodimer		CS	12p11 > qter (−)	
NAD-dependent isocitric dehydroge-nase (E.C. 1.1.1.41) Heterotetramer	α, β and γ subunits	IDH-M	α subunit 15q11 > qter (D15S114-D15S206) γ subunit Xqter (DXS1193-Xqter) (+; α and γ subunits)	
NADP-dependent isocitric dehydrogenase (E.C. 1.1.1.42 Homodimer		IDH2	15q21 > qter (+)	Homodimer; IDH1
Aconitase (E.C. 4.2.1.3) Monomer		ACO2	22q11 > q13 (D22S1171-D22S428) (−)	Monomomer, ACO1
α-ketoglutarate dehydrogenase	E1k (E.C. 1.2.4.2)	Oxoglutarate dehydrogenase, OGDH	7q13 > p11.2 (D7S667-D7S2427) additional copy: 10 (+)	
	E2k (E.C. 2.3.1.61)	Dihydrolipoyl succinyltransferase, DLST	14q24.2 > q24.3 (D14S71-D14S76) additional copy: 1p31 (+)	
	E3 (E.C. 1.8.1.4)	Dihydrolipoyl dehydrogenase DLD	7q31 > q32 (D7S2459-D7S692) (+)	
Succinyl-CoA synthetase GDP forming (EC 6.2.1.4) heterodimer	α and β subunits	succinate thiokinase succinyl CoA ligase	(β subunit) 13 (D13S263-D13S155) (−)	
Succinate dehydrogenase (EC 1.3.99.1) Heterodimer	iron-sulfur subunit	IP SDH, SDH1	1p35 > p36.1 (+)	
	flavoprotein	FP SDH, SDH2	5pter (D5S392) additional copy: 3q29	
Fumarate hydratase (E.C. 4.2.1.2)		fumarase, FH2	1q42.1 (D1S204) additional copies: 5 and 13	Homotetramer; FH1
NAD-dependent malate dehydrogenase (EC 1.1.1.37)		MDH2, M-MDH	7q13 > q22 (D7S675-D7S669) (−)	Homodimer; MDH1, C-MDH

Chromosomal locations were derived from release 96.0 of GenBank

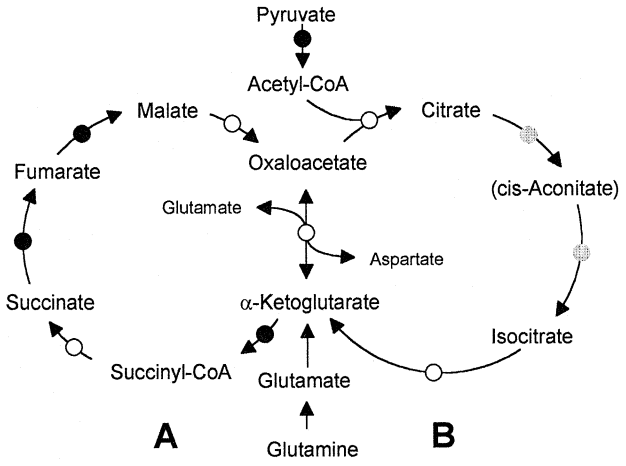

Fig. 2. Model for a functional splitting of the Krebs cycle reactions into complementary mini-cycles. The functioning of the first mini-cycle (A) would allow to convert pyruvate up to α-KG, even when the second mini-cycle (B) does not function. This could account for the urinary excretion of α-KG in patients presenting with defect of α-KG, SDH or fumarase activity. Similarly, it could produce reduced equivalents to sustain the normal oxygen uptake measured in circulating lymphocytes or cultured skin fibroblast from these patients.

in order to account for the complexity and the variety of the physiological contexts encountered in living cells. TCAC-related enzyme equipment of mitochondria may vary between species, and, in one given species, between organs. Thus mitochondria endowed with matrix malic enzyme can use malate as unique fuel molecule for TCAC running, malate being then used as a source of both pyruvate and oxaloacetate (OAA) through the reactions catalysed by the malic enzyme and the malate dehydrogenase respectively [7,8]. On the other hand, the unity of the cycle has been questioned and some suggestions have been made that it is actually comprised of 2 independent segments, allowing different fluxes, extending from α-KG to OAA and from OAA to α-KG, respectively [9]. Working in close connection with the aspartate-amino acid transferase (AAT, which produces α-KG), these would constitutes 'mini-Krebs cycles' consuming or producing glutamate and aspartate, requiring only catalytic amounts of OAA or of α-KG (Fig. 2).

The TCAC takes place in the semi-fluid matrix space of the mitochondria where metabolically-related enzymes appear to be associated into metabolons ensuring channelling of substrates through selected sets of enzymes [10]. Accordingly, the TCAC enzymes are consistently found in balanced proportions in different tissues [11], suggesting a concerted expression of the genes coding for TCAC enzymes. The semi-fluid state of the matrix also favours a kinetic compartmentation of soluble oxidation cofactors, such as nicotinamide adenine dinucleotides [12], together with the several dehydrogenases.

Citrate synthase, isocitrate and α-KG dehydrogenases are generally considered as important regulatory steps controlling the flux through the entire cycle [1]. The pyridine nucleotide redox poise (NADH/NAD+), the matrix phosphorylation potential (Pi + ADP/ATP ratio) and the Ca^{2+} concentrations act as major regulatory factors controlling several steps of the TCAC.

Finally, a wealth of evidences has been provided showing that thyroid hormones and glucocorticoids influence the activity of the TCAC cycle [13,14]. On one hand, thyroid hormone-induced increase of mitochondrial Ca^{2+} and substrate supply would stimulate the overall TCAC activity [13]. On the other hand, control of mitochondrial biogenesis by thyroid hormones and glucocorticoids, exerted at both transcriptional and translational levels, would account for broader term effect of hormones on TCAC activity [13,14].

The TCAC enzymes are all nuclearly-encoded. Most of the nuclear genes (11 on 15) encoding the protein moieties constitutive of the 9 TCAC mitochondrial enzymes have now been localized in man and 9 of the corresponding cDNAs have been cloned and sequenced (Table 1). Four of the TCAC enzymes possess both a mitochondrial and a cytosolic isoenzyme (Table 1). In the particular case of fumarase, both isoenzymes have been shown to be encoded by a single gene, which raises intriguing questions upon the mechanism controlling their sub-cellular distribution [15].

3. Krebs cycle diseases

Few cases of isolated and supposedly primary Krebs cycle disorders have been reported in human. Deficiencies of α-KG dehydrogenase, succinate de-

hydrogenase (SDH) and fumarase were reported in 3, 7 and 14 patients, respectively (Table 2). Beside these specific enzyme defects, enzymes of the TCAC can be affected concurrently with enzymes involved in other metabolic pathways. Thus, a defect of the dihydrolipoamide dehydrogenase (the E_3 component of the 2-ketoacid dehydrogenases) characteristically associates a defective activity of the pyruvate and the branched-chain amino acid dehydrogenases together with a decreased α-KG dehydrogenase activity [33]. A decrease of both aconitase and SDH has also been reported in one patient presenting with generalized defect of iron-sulfur proteins, including rhodanese, the Rieske protein of the complex III and several subunits of the complex I of the respiratory chain [34].

Among the major clinical features encountered in patients presenting isolated TCAC enzyme defects, neurological impairments - associated or not with muscular involvement - are prominent figures, being encountered in 19/22 cases (85%), with encephalopathy (11 cases) and typical Leigh syndrome (1 case) being reported in 70% of the documented cases (Table 2). Hypertrophic cardiomyopathy, isolated (1 case) or part of a pluritissular disease (4 cases), has also been reported in 18% of the cases, all of them presenting with a SDH deficiency, except for one case presenting with α-KG dehydrogenase deficiency [17]. Interestingly enough, no cardiac involvement was reported for any of the patients presenting with a fumarase deficiency (Table 2).

The age of onset of fumarase and α-KG dehydrogenase deficiencies was consistently below 1 y of age, with hypotonia, failure to thrive and/or lactic acidosis being frequently first noticed. In contrast, patients with SDH deficiencies were often diagnosed after several years of life [19] or even in adulthood [20,23]. Onset symptoms associated with SDH deficiencies vary from case to case, among them growth retardation, pulmonary oedema, bronchiolitis, body rigidity or optic atrophy. Although not consistently, the latter onset and the milder outcome noticed for SDH-deficient patients correspond to relatively higher enzyme residual activities (Table 2).

An abnormal urinary excretion of organic acids was frequently noticed in those patients with TCAC enzyme deficiencies, with occasional peaks of α-KG observed whatever the enzyme deficiency (α-KG dehydrogenase, SDH, or fumarase). Obviously due to the enzyme defect in cases of α-KG dehydrogenase deficiencies, this α-KG excretion might result from the secondary blockade of this enzyme by succinyl CoA in the case of SDH and fumarase deficiencies (see Fig. 1). The excretion of α-KG is also an indication that at least a segment of the TCAC metabolizes pyruvate. This possibly accounts as well for the inconsistency of the lactic acidosis and the mild, if any, elevation of L/P ratios measured in these patients.

4. Biochemical, molecular findings and genetics

As mentioned above, while profound defects of fumarase (both cytosolic and mitochondrial isoenzymes) and α-KG dehydrogenase have often been reported (less than 1% in vitro residual activity), only partial SDH defects have been reported (more than 25% in vitro residual activity). Incidentally, it is noteworthy that the profound defects of TCAC enzymes only concern enzymes involved in one of the mini-Krebs cycle suggesting an activation of an in vivo metabolic by-pass allowing cell survival (Fig. 2). Accordingly, normal respiration rates have been consistently observed in the enzyme deficient cells (circulating lymphocytes and cultured skin fibroblasts) of these patients [3,35].

SDH deficiencies are also distinct by their variable tissue-specific expression which was observed in three of the cases reported [21,23,24]. This was somewhat surprising considering that the products of the house-keeping TCAC genes are essentially distributed among all human tissues. No functional additional copies of Krebs cycle genes have been reported so far, suggesting that the tissue-specific expression pattern should rather be ascribed to tissue-specific regulations of transcriptional and/or translational activities.

Identification of mutations causing TCAC enzyme deficiencies have been now reported for both fumarase [3,34] and SDH [4]. A mutation in the fumarase cDNA was first reported in two siblings with progressive encephalopathy and fumarase deficiency [3]. Both patients were found homozygous for a missense mutation, a G955C transversion, predicting a Glu319Gln substitution in a highly conserved do-

Table 2
Clinical, metabolic, biochemical and molecular findings in patients presenting with isolated Krebs cycle enzyme and defects

Disorder (occurrence)	Sex	Onset symptom	Clinical features	Residual enzyme activity	Outcome	Lactic acidosis	Urinary metabolites	Heredity	Molecular bases	Ref.	Comments
α-Keto-glutarate dehydrogenase deficiency (3 cases)	5 M, 2 F										
Case 1	M	hypotonia, 16 mth	hypotonia, involontary movements, neurodegenerative condition	≈ 25% (fibro)	alive, 3 y	slightly elevated lactatemia and L/P, slightly elevated lactato racchia with normal L/P	elevated α-ketoglutarate	consanguineous parents	nd	[16]	subunit E2 suspected
	F	muscular rigidity, 18 mth		≈ 25% (fibro)	alive, 3 y	normal lactatemia and lactatoracchia	elevated α-ketoglutarate	*id*	nd	[16]	*id*.
Case 2 (3 brothers)	M	congenital lactic acidosis	hypotonia, cortical atrophy, hypertrophic cardiomyopathy	< 1% (fibro)	† 32 mo	elevated lactatemia and L/P	occasional α-ketoglutarate peaks	consanguineous parents	nd	[17]	low plasma hydroxybutyrate/acetoacetate ratio
	M	congenital lactic acidosis	severe hypotonia, tachypnea	< 1% (fibro)	† 30 mo	elevated lactatemia and L/P	occasional α-ketoglutarate, succinate, fumarate and malate peaks	*id*.	nd	[17]	*id*.
	M	congenital lactic acidosis	mild truncal hypotnia, neurodegenerative condition	< 1% (fibro)	alive, 20 mo	elevated lactatemia and L/P	occasional α-ketoglutarate peaks	*id*.	nd	[17]	*id*.

Case 3 (2 siblings)	M	hypotonia	hypotonia, progressive encephalopathy, metabolic acidosis	7.5% (muscle) 15% (fibro)	† 10 y	elevated lactatemia and L/P	occasional α-ketoglutarate peaks	consanguineous parents	nd	[18]	subunit E2 suspected; inconsistently low hydroxybutyrate/acetoacetate ratio
	F	congenital lactic acidosis	hypotonia, encephalopathy, mild permanent metabolic acidosis	14% (fibro)	alive, 4 y	inconsistent elevation of lactatemia with mild elevation of L/P	occasional α-ketoglutarate peaks	*id.*	nd	[18]	*id.*
Succinate dehydrogenase deficiency (7 cases)	4 M, 6 F										
Case 1	F	growth retardation, 6 y	Kearns-Sayre syndrome (external ophthalmoplegia, short stature, ataxia, cardiac conduction defect, pigmentary retinopathy)	≈ 10% (muscle)	alive, 25 y	elevated lactatemia and L/P		no consanguinity	nd	[19]	9 non affected siblings
	M	epileptic fits, 8 mth	hypertrophic cardiomyopathy, skeletal muscle myopathy	≈ 30% (muscle)	alive, 19 y			*id.*	*id.*	[20]	
Case 3	F	severe bronchiolitis, 5 mth	isolated hypertrophic cardiomyopathy	≈ 25% (heart)	† 8 mo	normal lactatemia and L/P		no consanguinity	nd	[21]	isolated heart involvement; no deficiency in muscle, liver, leuco, fibro)

Table 2 (continued)

Disorder (occurrence)	Sex	Onset symptom	Clinical features	Residual enzyme activity	Outcome	Lactic acidosis	Urinary metabolites	Heredity	Molecular bases	Ref.	Comments
Case 4 (2 brothers)	M	left side body rigidity, 10 mth	leukodystrophy with Leigh syndrome	≈ 40% (leuco, fibro, muscle)	† 19 mo	mild lactatemia, normal lactatoracchia	peaks of α-ketoglutarate	consanguineous parents	Arg544Trp (SDH Fp gene)	[4]	1 healthy sister; 1 affected foetus
	M	marked rigidity 10 mth	leukodystrophy with Leigh syndrome	≈ 40% (leuco, fibro, muscle)	† 24 mo	mild lactatemia, mild lactatoracchia	peaks of α-ketoglutarate and succinate	*id.*	Arg544Trp (SDH Fp gene)	[4]	*id.*
Case 5	F	proximal muscle weakness, myalgia and dysphagia	polymyositis with sarcoidosis	< 50% (muscle)	alive, 33 y					[22]	
Case 6	F	late onset optic atrophy	cerebellar ataxia	50% (muscle, platelets)	alive, 55 y				nd	[23]	no deficiency in cultured cells (fibro and lymphoblasts)
	F	late onset optic atrophy	cerebellar ataxia	50% (muscle, platelets)					nd	[23]	

Case 7	F	psychomotor regression 10 mth	leukodystrophy with Leigh syndrome	40% (muscle)	alive, 5 y	elevated lactatemia		no consanguinity	nd	[24]
Fumarase deficiency (14 cases)	6 M, 4 F									
Case 1	M	cyanotic spells, hypothermia, 1 day	encephalomyopathy with failure to thrive, developmental delay, hypotonia, cerebral atrophy	< 2% (liver, muscle)	† 8 mo	elevated lactatemia and L/P	peaks of fumarate, succinate and citrate	no consanguinity	nd	[25]
Case 2	M	partial seizure 10 wk	encephalomyopathy with marked hypotnia, microcephaly, delayed psychomotor development, cerebral atrophy	< 5% (heart, liver, kidney, brain and cerebellar cortex, fibro, muscle)	† 7 mo	elevated lactatemia	peaks of lactate, pyruvate, fumarate and succinate; terminally, peak of α-ketoglutarate	no consanguinity	3 bp insertion (Lys434ins) and a Arg190Cys substitution	[26]
Case 3	M	small head size, hypotonia, 6 mth	hypotonia, microcephaly delayed development	< 25% (fibro)			peaks of fumarate and succinate	consanguineous parents	nd	[27]

1 brother healthy; both cytosolic and mitochondrial enzymes deficient	
both cytosolic and mitochondrial enzymes deficient	
1 healthy brother; both cytosolic and mitochondrial enzymes deficient	

Table 2 (continued)

Disorder (occurrence)	Sex	Onset symptom	Clinical features	Residual enzyme activity	Outcome	Lactic acidosis	Urinary metabolites	Heredity	Molecular bases	Ref.	Comments
Case 4 (2 brothers)	M	polyhydramnios, enlarged cerebral ventricles in utero	encephalomyopathy with cerebral atrophy, severe developmental delay, infantile spasms, hypsarythmia	< 0.5% (leuco, fibro)	† 5 yr		peaks of fumarate	no consanguinity	nd	[28]	three non affected siblings
	M	polyhydramnios, enlarged cerebral ventricles in utero	encephalomyopathy with cerebral atrophy, severe developmental delay, infantile spasms, hypsarythmia		† 3 yr		peaks of fumarate	*id.*	nd	[28]	
Case 5	F	congenital hypotonia	static encephalopathy with failure to thrive, psychomotor developmental delay, mild diffuse cortical atrophy	10% (fibro)	alive, 5 yr	normal lactatemia	peaks of fumarate	no consanguinity	nd	[29]	two healthy sisters
Case 6	F	dysmorphic facial features, hydramnios, 9 mth	encephalopathy with corpus callosum agenesis moderate communicating hydrocephalus, liver involvement with bile and lipochrome pigment accumulation in Kupffer cells	< 15% (liver)	† 6 mth	raised blood pyruvate	peaks of fumarate, α-ketoglutarate; terminally, peaks of succinate and lactate	consanguineous parents	nd	[30]	one healthy sibling
Case 7 (2 sisters)	F	hypertonic limb movements, 1 mth	progressive encephalopathy with severe mental retardation, severe microcepahly neutropenia	< 1% (leuco, liver, muscle, fibro)	alive, 7 yr	normal lactatemia and L/P, elevated lactatoracchia	peaks of fumaricacid, α-ketoglutarate	consanguineous parents heterozygous for the G-955 > C mutation	Glu-319Gln	[3]	two dead neonate siblings, one brother and two sisters healthy

	F	poor sucking, hypertonic episodes of upper limbs, 2 mth	progressive encephalopathy with moderate microcephaly, developmental delay, severe neutropenia	< 1% (leuco, muscle, fibro)	alive, 3 y	normal lactatemia and L/P, elevated lactatoracchia	peaks of fumaricacid, α-ketoglutarate	*id.*	Glu319Gln	[3]	*id.*
Case 8	M	poor sucking floppiness at birth	encephalopathy with non progressive hypotonia and developmental delayed	< 1% (fibro)	alive, 6 yr	occasional raised blood lactate and pyruvate, elevated L/P	occasional peaks of fumaric acid	consanguineous parents	nd	[31]	50% fumarase activity in both parent's cultured skin fibroblasts
Case 9–14		failure to thrive	developmental delay, hypotonia	10 to 20%				2/5 patients with consanguineous parents	- 75 bp deletion, - bp insertion, - 4 substitutiuons; Ala265Thr; Asp383Val; PHe269Cys; Trp458Stop	[32]	
Prevalent features of TCAC-enzyme deficiencies (24 cases)	15 M, 12 F	various	neurological impairment and/or muscular involvement and/or heart involvement	< 1 to 50% residual activities	inconsistent lactacidosis and elevated L/P ratios	inconsistent peaks of α-keto-glutarate	molecular bases identified in 7/24 cases				

main of the protein. Both first cousin parents were found heterozygous for this substitution. Accordingly, fumarase activity in parent's lymphoblastoid cell lines represented about 50% of mean control activity, in both the mitochondrial and cytosolic compartments. Mutations of the fumarase cDNA were since reported in six unrelated families (Table 2).

A mutation in the succinate flavoprotein subunit cDNA has been reported in two sisters presenting with Leigh syndrome and SDH deficiency [3]. Both patients were found homozygous for a Arg544Trp substitution in a highly conserved domain of the SDH cDNA. Both consanguineous parents were found heterozygous for this substitution. Expression of mutant and wild-type SDH Fp cDNAs in enzyme-deficient yeast strain confirmed the pathogenic character of this substitution.

Our present knowledge on the molecular bases of TCAC diseases is restricted to the cases mentioned above.

Recessive inheritance of the mutations was noticed in both of the cases of fumarase and SDH deficiencies which have been elucidated [2,3]. The consanguinity of the parents suggests a similar recessive inheritance in the cases of α-KG dehydrogenase and in most cases of fumarase deficiencies [16–18,34]. Several patients presenting with SDH or fumarase defect and born from unrelated parents have also been reported [19–21,25,26,28,29]. Nevertheless, the primary nature of these defects remains to be established before assessing other modes of inheritances.

5. Conclusion

The recent elucidation of the molecular basis of several TCAC enzyme deficiencies definitively establishes the occurrence of isolated and primary TCAC deficiencies in man. This new group of diseases has to be added to other known diseases affecting the oxidative activities of the mitochondria, namely respiratory chain activity and fatty acid β-oxidation. No clinical clue allows to easily distinguish between these different diseases, the patients often presenting with similar neuromuscular impairments. The disclosure of anomalous urinary excretion of specific organic acids should however prompt investigation of TCAC enzyme activities. Noticeably, an excretion of organic acids, particularly of α-KG, is also frequently observed in respiratory chain disorders, but this generally does not specifically concern one unique metabolite.

The investigation of the patients presenting with a TCAC enzyme defect has revealed a surprising complexity, the mechanism of which remains largely to be established. Already, it teaches us that, as previously suggested in animal [9], the TCAC in human is possibly organized around two distinct cycles, one of which being potentially by-passed in vivo. This probably accounts for the normal respiration and survival of enzyme-deficient cells in culture, as well as for the several-years survival of most of these patients, despite a life-threatening prognosis.

Acknowledgements

We would like to thank J. Bastin and F. Djouadji for helpful discussions and C. Mussou for help in preparing the manuscript.

References

[1] Tyler, D. (1992) in The mitochondrion in Health and Disease, VCH publishers.
[2] D.T. Whelan, R.E. Hill, S. McClorry, Clin. Chim. Acta 132 (1983) 301–308.
[3] T. Bourgeron et al., J. Clin. Invest. 93 (1994) 2514–2518.
[4] T. Bourgeron et al., Nature Genet. 11 (1995) 144–149.
[5] H.A. Krebs, W.A. Johnson, Enzymologia 4 (1937) 148–156.
[6] W. Wieser, Trends Biochem. Sci. 5 (1980) 284.
[7] J.M. Palmer, Ann. Rev. Plant Physiol. 27 (1976) 133–157.
[8] C. Lance, P. Rustin, Physiol. Veg. 22 (1984) 625–641.
[9] M. Yudkoff, J. Biol. Chem. 269 (1994) 27414–27420.
[10] P.A. Srere, Trends Biochem. Sci. 10 (1985) 109–110.
[11] D. Pette, Regulation of metabolic processes in mitochondria, 1966, Elsevier, Amsterdam.
[12] P. Rustin, et al. Plant mitochondria. Structural, functional and physiological aspects, 1987, Plenum Press, New York.
[13] S. Soboll, Biochim. Biophys. Acta 1144 (1993) 1–16.
[14] F. Djouadi, A. Wijkhuisen, J. Bastin, Am. J. Physiol. 263 (Renal Fluid Electrolyte Physiol. 32) 1982, F237-F242.
[15] T. Suzuki et al., J. Biol. Chem. 264 (1989) 2581–2586.
[16] A. Kohlschütter et al., Eur. J. Pediatr. 138 (1982) 32–37.
[17] J.P. Bonnefont et al., J. Pediatr. 121 (1992) 255–258.
[18] N. Guffon et al., J. Inher. Metab. Dis. 16 (1993) 821–830.
[19] M.D. Rivner et al., Neurology 39 (1989) 693–696.
[20] H. Reichman, C. Angelini, Eur. Neurol. 34 (1993) 95–98.
[21] P. Rustin et al., J. Inher. Metab. Dis. 16 (1993) 541–544.

[22] H. Reichmann et al., Neuromusc. Dis. 5 (1995) 277–283.
[23] Taylor, R.W. et al. (1995) SSIEM abstract, pp. 69.
[24] M.A. Birch-Machin et al., Biochem. Biophys. Res. Commun. 220 (1996) 57–62.
[25] A.B. Zinn, D.S. Keer, C.L. Hoopel, N. Eng. J. Med. 315 (1986) 469–475.
[26] C. Gellera et al., Neurology 40 (1990) 495–499.
[27] R. Petrova-Benedict et al., Am. J. Hum. Genet. 40 (1987) 257–266.
[28] A.M. Remes et al., Pediatr. 89 (1992) 730–734.
[29] O.N. Elpeleg, N. Amir, E. Christensen, J. Pediatr. 121 (1992) 752–754.
[30] V. Walker et al., J. Inher. Metab. Dis. 12 (1989) 331–332.
[31] V. Narayanam, W. Diven, M. Ahdad-Barmada, J. Child Neurol. 11 (1996) 252–255.
[32] Shih, V.E. et al. (1995) SSIEM abstract, pp. 69.
[33] B.H. Robinson, J. Bioenerg. Biomembr. 20 (1988) 313–323.
[34] R.E. Hall et al., J. Clin. Invest. 92 (1993) 2660–2666.
[35] M. Bourgeois et al., Brain Dev. 14 (1992) 404–408.